Hybridization may promote variation in cognitive phenotypes in experimental guppy hybrids

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Abstract

Hybridization is an underappreciated mechanism of evolution. While hybrids often express inferior traits and are selected against, hybridization can promote phenotypic variation and produce trait combinations distinct from the parentals, generating novel adaptive potential. Among other traits, hybridization can impact behaviour and cognition and may reinforce species boundaries when hybrids show decreased cognitive abilities. However, the hypothesized role of hybridization in the diversification of cognitive phenotypes remains enigmatic. To test this idea, we compare the performance of guppies (Poecilia reticulata), Endler's guppies (Poecilia wingei), and their experimental hybrids in colour association and reversal learning. In addition, we introduce a new approach to compare multidimensional cognitive phenotypes. We found that hybrids showed intermediate learning abilities in both tasks compared to the parentals. Moreover, hybrids had slightly higher phenotypic dispersion, new trait combinations occurred in some hybrid individuals, and the mean phenotype of one hybrid group deviated away from the axis of variation of the parentals. Our method should hence be useful in further exploring how hybridization, and other evolutionary processes, impact behavioural and cognitive traits. Our results suggest that hybridization may promote cognitive variation and generate new trait combinations, even when learning performance at the group level is intermediate between parentals.

Keywords

Transgressive segregation; phenotypic novelty; kernel density estimation; associative learning; cognitive flexibility

1. Introduction

Interspecific hybridization often leads to hybrids that are less fit than the two parental species [1, 2]. Such fitness costs come from hybrid inviability or infertility [3-5], but also from physiological or behavioural phenotypes that are impaired or ecologically mismatched. For example, hybrids of *Drosophila* have decreased foraging efficiency [6] and hybrids of migratory fish that express intermediate migratory strategies have higher predation rates [7]. In these cases, intrinsic (genetic) and extrinsic (ecological) selection against hybrids can become a postzygotic isolating barrier that reinforces species boundaries [1]. On the other hand, there is now widespread evidence that hybridization can also be a catalyst of phenotypic variation, leading to ecological and evolutionary innovations [8-10]. Through recombination of parental alleles, hybridization can generate high levels of heritable variation in hybrids, including individuals that express new trait combinations that exceed the range of both parental species, referred to as transgressive [11, 12]. Under certain ecological conditions, such variation may provide hybrids with opportunity for the evolution of novel adaptations or to explore underused niches [8, 13]. For example, transgression in bill morphology allowed the hybrids of two Galapagos finches to forage on novel food items and led to the formation of a new species [14]. Despite an apparent contrast, the ideas of hybridization resulting in unfit hybrids that are selected against or in transgressive hybrids with evolutionary novelty potential are not mutually exclusive. Even if most of the variation produced is deleterious, a few rare transgressive individuals can become established if intrinsic selection is not strong and extrinsic selection is favourable [11, 15]. For instance, early hybrids of the *Tigriopus* copepod show reduced survival during development, but a few generations of recombination are enough to recover fitness to the levels of the parentals. At the same time, some of these hybrid lineages can thrive in extreme temperatures that are lethal to the parental species, and thus may become established in these unused niches [15].

Transgressive phenotypes are common in both plants and animals, and different mechanisms have been proposed to explain how recombination of parental genomes can result in new traits [16-19]. When alleles have complementary additive effects, first-generation (F1) hybrids can only express intermediate phenotypes, but effects may sum in the F2 and higher generations and lead to transgression [17]. By contrast, extreme hybrid phenotypes can arise in F1 hybrids through dominance of some loci contributing to variation in the same trait or epistatic interactions of alleles at different loci, among other mechanisms [16, 18, 20]. More inclusive definitions of transgression may also encompass ecological or functional transgression, not classically considered a transgressive effect from a genetic point of view [11, 21]. In these cases, novel hybrid variation may arise from new combinations of traits that fall between disjunct parental ranges, which can perform new functions or facilitate expansion into new areas of functional space.

One way in which hybridization can influence the trajectories of species is through effects on behavioural phenotypes. When hybrids show potentially maladaptive behaviours, such as impaired foraging, reproductive, or migratory behaviours, then behavioural traits can contribute to selection against hybrids and play a role as reproductive isolation barriers [6, 7, 22, 23]. However, hybridization can also lead to behavioural novelty. Even if hybrid individuals show intermediate or poorer behaviours in the parental species niches, for example lower feeding efficiency on parental food types, they may have higher efficiency on novel food types and therefore be able to exploit resources outside the parental species niche, as observed in hybrids of African cichlids [24]. In addition, behavioural novelty can indirectly arise from transgression in other traits, such as novel combinations of morphological characters that may allow the expression of new behaviours [25].

Similar to behavioural phenotypes, recent studies have proposed a role for cognitive abilities such as learning and memory in influencing the outcome of hybridization events [26, 27]. Hybrids of black-capped (*Poecile atricapillus*) and Carolina (*P. carolinensis*) chickadees performed worse in a spatial task mimicking seed caching and were less likely to solve a novel problem than their parental species [28]. Since these birds rely on scatter-hoarding to survive harsh winters, hybrids showing poor learning and memory may suffer a fitness disadvantage in the wild [28]. In other cases, better learning and memory scores have been reported in F1 hybrids of domesticated mice and of horses and donkeys (mules); such cases are likely a result of heterosis, i.e., enhanced heterozygosity from outbreeding domesticated species [29-31]. If heterosis underlies enhanced cognitive abilities, this should be a transient effect restricted to F1 hybrids [32]. Surprisingly, one complementary outcome of hybridization on cognition that may also influence ecological selection on hybrids remains to be tested. Hybridization may increase cognitive phenotypic variation in hybrids and promote transgression in cognitive performance. Here we test this hypothesis.

By measuring the learning performance of hybrids between guppies (*Poecilia reticulata*) and Endler's guppies (*Poecilia wingei*) and the parental species in two cognitive tasks, we examine how the learning abilities of hybrids compare to the parentals and test whether hybrids display larger variation and transgression in learning performance. Specifically, we assess individual performance on two different cognitive tests – a colour association task testing simple learning abilities and a reversal learning task testing cognitive flexibility. In addition to differing in complexity, the two cognitive traits assayed by these tasks are encoded by different brain regions and may represent different trade-offs between costs and benefits of investment in each cognitive trait [26, 33-35]. To characterise phenotypic dispersion and assess the occurrence of transgression, we use a novel approach that combines the learning scores of the two tasks into clusters of cognitive phenotypes. Borrowing from methods used in movement ecology and ecological niche analyses [36, 37], together with a geometric approach to quantify patterns of hybrid phenotypes [18, 20], we quantitatively compare phenotypic dispersion between hybrids and parentals and quantify the extent to which hybrids are transgressive and/or deviate from parental mean phenotypes. If hybrids perform worse in these cognitive tests compared to one or both parental species, then these traits may represent a postzygotic isolating barrier against hybridization [27]. However, if hybrid phenotypes are over-dispersed relative to parents or deviate from parental mean phenotypes, then those hybrids may have cognitive innovation potential [13, 18].

2. Methods

2.1. Parental species and F1 hybrid breeding

Parental fish used for breeding derived from laboratory populations kept in identical conditions at Wageningen University & Research. Common guppy (*Poecilia reticulata*) populations descend from wild individuals caught in the upper Aripo river, Trinidad, and Endler's guppy (*P. wingei*) populations descend from wild individuals from Cumaná, Venezuela. Experimental fish were bred and tested for cognitive ability in two blocks. In each block, two reciprocal first-generation (F1) hybrid crosses (i.e., *P. reticulata* females crossed with *P. wingei* males, and *P. wingei* females crossed with *P. reticulata* males) and non-hybrid (parental) crosses were bred and raised simultaneously. We will refer to the parental species as R for *P.* ***r****eticulata* and W for *P.* ***w****ingei* and use these abbreviations for the hybrid crosses as mother species × father species (i.e., R×W and W×R). All aquaria were part of a recirculation system, in which water chemistry, temperature, light conditions, and feeding regime were kept constant. All fish were bred and raised in identical conditions in a common garden environment (for details on the breeding and rearing of the parental species and F1 hybrids see supplementary material).

2.2. Learning assays

Individuals used in the cognitive assays were adult females, at an approximate age of six months. Only females were tested as males are often difficult to motivate with a food reward [38]. The fish were tested in two blocks in 60 experimental tanks, where they were individually housed for the duration of the learning tasks. In each replicate, females from the two F1 hybrid crosses and the two parental crosses were assigned a unique identifier and randomly distributed across the 60 tanks, and the experiments were run blind to the group of each animal. The experimental tanks included a home compartment (25×15 cm) and an experimental compartment (15×15 cm) at the front of the tank, only accessible during training sessions through a guillotine door (Fig. 1; see supplementary material for additional details). Fish were confined to the home compartment outside of training sessions, where they had visual contact with fish in neighbouring tanks to prevent social isolation. The experimental compartment, however, was visually isolated to avoid social learning effects.

To test for associative and reversal learning, we used a well-established colour discrimination assay for fish, using red and yellow as stimulus colours [38, 39]. Before the start of the experiment, fish were haphazardly assigned to either red or yellow as the correct stimulus, balanced across the four groups. The experimental compartment contained a white plate with 20 identical circular holes (5 mm deep, 10 mm diameter).

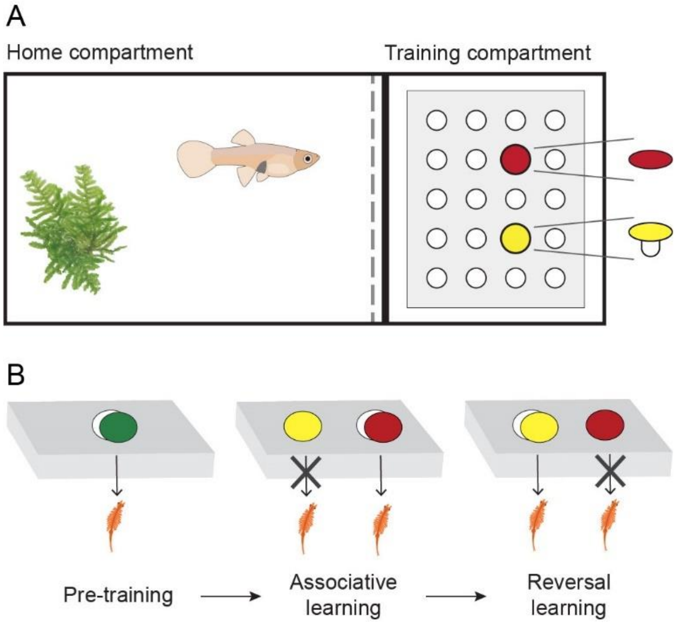

Figure 1. Schematic representation of the experimental tank and cognitive assays. (a) The experimental tank consisted of a home compartment and a training compartment. (b) In pre-training, fish were taught to dislodge a green disc to access a food reward (one brine shrimp) in a hole underneath. During the learning tasks, fish were given a choice between two coloured discs, both concealing a food reward. The negative stimulus was fixed in the hole with a plastic knob and could not be moved by the fish to uncover the food item beneath it.

2.2.1. Pre-training

During pre-training trials, fish learned to dislodge a green disc to access a food reward (one frozen *Artemia*) hidden in one of the holes. The trial started with the opening of the opaque door; 5 s later, we opened the transparent door. The fish could then voluntarily enter the training compartment and find the food reward. During the first trials, the disc only partially covered the hole, leaving the reward exposed. We then trained the fish to dislodge the green disc by successively moving the disc from partially to fully covering the hole. Six females (R, n=2; W, n = 1; R×W, n=2; W×R, n=1) did not feed during early pre-training stages and were replaced by new fish. A further seven females (R, n=3; R×W, n=3; W×R, n=1) did not dislodge the disc during late pre-training stages and were excluded from the experiment. Fish ran on average a total of 33 pre-training trials (additional details in supplementary material). A total of 113 females (R, n=27; W, n = 30; R×W, n=27; W×R, n=29) succeeded to dislodge the disc in all trials of the last two days of pre-training and continued the experiment.

2.2.2. Associative learning

In the associative learning task, fish were given a choice between a red and a yellow disc, both concealing a food reward (to ensure fish could not be learning through olfactory cues). Only one of the discs could be dislodged by the fish to reveal a reward (positive stimulus), as the negative stimulus was kept in the hole with a plastic knob and could not be moved to uncover the food item beneath it. For each trial, we randomised the position (left/right) of the correct colour, with the constraint of no more than two consecutive trials in the same position, to avoid side biases. Choice was recorded as the first disc the fish touched. The fish was given 1 min to dislodge the correct coloured disc and eat the reward. For incorrect trials, correction was allowed within 3 min. If the fish failed to correct its choice within 3 min, and when fish failed to make a choice within 1min, we moved the rewarded disc 5 mm to the side to allow easy access to the food. This ensured that all fish experienced the same number of reinforced trials throughout the experiment. Training for the colour discrimination task comprised a minimum of 12 trials for all fish. The learning criterion consisted of 7 out of 7 correct choices (significant according to a binomial probability). As soon as a subject reached the learning criterion, the next phase commenced. If a subject did not reach the learning criterion within 40 trials, it was excluded from further training.

2.2.3. Reversal learning

After passing the learning criterion, the reversal learning task started. The procedure was the same as the during the associative learning phase except the reward contingency was reversed: fish previously trained on yellow were trained on red and vice-versa (Fig. 1). Each subject ran a minimum of 24 training trials and continued the reversal task until it reached the ‘7 out of 7’ learning criterion, up to a maximum of 72 trials.

2.3. Analysis of learning performance

Out of 113 females that completed the cognitive assays, three females (R, n=2; R×W, n=1) were excluded from the analysis due to repeated refusal to dislodge the discs (< 40% recorded choices during training trials). Statistical analyses were performed in R v.3.6.3 [40] using ‘*lme4’* [41]. For both cognitive tasks, we compared the performance of the parental species and F1 hybrids in (i) number of trials to reach learning criterion using a generalised linear model (Poisson distribution) with Crossing Group (R, W, R×W, W×R), Colour (red, yellow), and Testing Block (block 1, block 2) as potential predictor variables; and (ii) learning rate, i.e., probability of success per trial (correct = 1; incorrect = 0) using a generalised linear mixed-effect model (binomial distribution) with Trial Number, Crossing Group, Colour, Testing Block, and the interaction of Trial Number × Crossing Group and Trial Number × Colour as predictor variables, as well as a random intercept and slope for Fish Identity, which accounts for the repeated observations of individual fish. If Crossing Group was a significant predictor in the model, we assessed Tukey corrected multiple comparisons between

Crossing levels using the '*glht'* function in the *'multcomp'* package [42]. We tested the significance of the random effects in (ii) models with likelihood ratio tests, by comparing models which culled the intercept or slope term to our final model. To examine if performance in the associative learning task had carryover effects on performance in the reversal task, we tested the inclusion of the predictor 'Trials to learning criterion in the associative learning task' and its interaction with Crossing Group in models (i, ii) for the reversal task. For model (ii) in the reversal task, Trial Number was log-transformed to meet the assumption of linearity on the logit-scale.

2.4. Analysis of transgressive trait variation in hybrids

2.4.1. Quantifying phenotypic dispersion

To quantify phenotypic dispersion, we used Kernel Density Estimation (KDE), an established method for probability density estimation that is commonly used in ecology [e.g. home range, morphological, or ecological niche analyses; 36, 37]. From individual scores in the associative and reversal learning tasks (number of trials to reach learning criterion), KDE allowed us to estimate the clusters of 'cognitive space' occupied by each group across the two dimensions of learning performance. KDE clusters were computed with the *'kde'* function in the *ks* package [43], with the bandwidth estimated using a grid-search estimation. The number of trials to reach criterion was log-transformed to approximate a continuous variable with Gaussian distribution. Phenotypic dispersion was estimated as the hypervolume of the KDE cluster that contains 95% of the individuals in the group with the '*contourSizes'* function in the *ks* package [20].

2.4.2. Identifying transgressive hybrids

We considered hybrid phenotypes to be transgressive if their trait values fell outside the range of both parental species [11, 16], i.e., outside the 95% KDE of both parents. For each hybrid group, we quantified the frequency and proportion of transgressive individuals.

2.4.3. Comparing mean hybrid phenotype to simulated hybrid populations

Under an expectation of complete additive inheritance, F1 hybrids should be a linear combination of the parental phenotypes [18]. To test whether the mean phenotypes of R×W and W×R hybrids deviate from the expected intermediate trait value, we first simulated hybrid phenotypes as a linear combination of our parental individuals. Simulated hybrids were generated by randomly sampling a pair of individuals from each parental species and calculating the mean trait values of the parental pair. To approximate the sample size of our experimental groups, we used 30 randomly chosen parents from each species to create 30 simulated hybrids and repeated this process 100 times. We then compared the observed trait values of R×W and W×R hybrids to the simulated intermediate hybrid populations with regards to two metrics: 'parental bias', which captures deviation of hybrid phenotypes from the arithmetic mean of the parental phenotypes in the direction of either parent [18, 20]; and ‘phenotypic mismatch’, which captures the deviation of the hybrid mean phenotype away from the line connecting parental mean phenotypes [18, 20]. The two metrics were estimated by considering the geometric position of the mean phenotypes of the two parents and the hybrid. ‘Parental bias’ is given by the position of the hybrid projection on the line connecting parental mean phenotypes (where a value of zero corresponds to a hybrid phenotype that is exactly intermediate), while ‘parental mismatch’ corresponds to the Euclidian distance of the average hybrid phenotype to the line drawn between the two parental means (where a minimum value of zero corresponds to a hybrid phenotype that falls on the line connecting the parents).

3. Results

3.1. Do parental species and hybrids differ in learning performance?

In the associative learning task, common guppies (R) had the lowest success rate, with 20/27 (74%) R females reaching the learning criterion. The two F1 hybrid groups had high success rates similar to Endler’s guppies (W): 26/27 (96%) R×W females, 28/29 (97%) W×R females, and 30/30 (100%) W females reached learning criterion. When comparing the number of trials needed to learn the colour association, Endler’s guppies succeeded in the task faster than common guppies and their hybrids (Fig.2a; Tukey post-hoc tests, all P<0.001; Table S1a; Table S2). All four groups improved their success rate as the task progressed, indicating they succeeded in learning the task. Endler’s guppies and W×R hybrids showed a steeper learning curve compared to the other two groups (Fig.2b; Table S1b;). The number of trials to learn and success rate differed between testing blocks as in the second block the difference in performance between groups was not as strong (Table S1a,b). Individual fish differed in their naïve probability of choosing the rewarded colour in the first trial (random intercept for Fish Identity; Table S1b) and in their speed of acquisition over trials (random slope for Fish Identity; Table S1b).

In the reversal learning task, success rates were also very high with 19/19 (100%) R females, 24/26 (92%) R×W females, 28/28 (100%) W×R females, and 30/30 (100%) W females reaching learning criterion. Female Endler’s guppies and R×W hybrids needed fewer trials to learn the colour reversal compared to common guppies and W×R hybrids (Fig.2c; Tukey post-hoc tests, all P<0.02; Table S1c; Table S3). All four groups improved their success rate as the task progressed, with female Endler’s guppies showing a steeper learning curve compared to the other groups (Fig.2d; Table S1d). Performance in the associative learning task had a marginal positive effect on success in the reversal task; fish that were faster in learning the initial association had higher success rates (Table S1d).

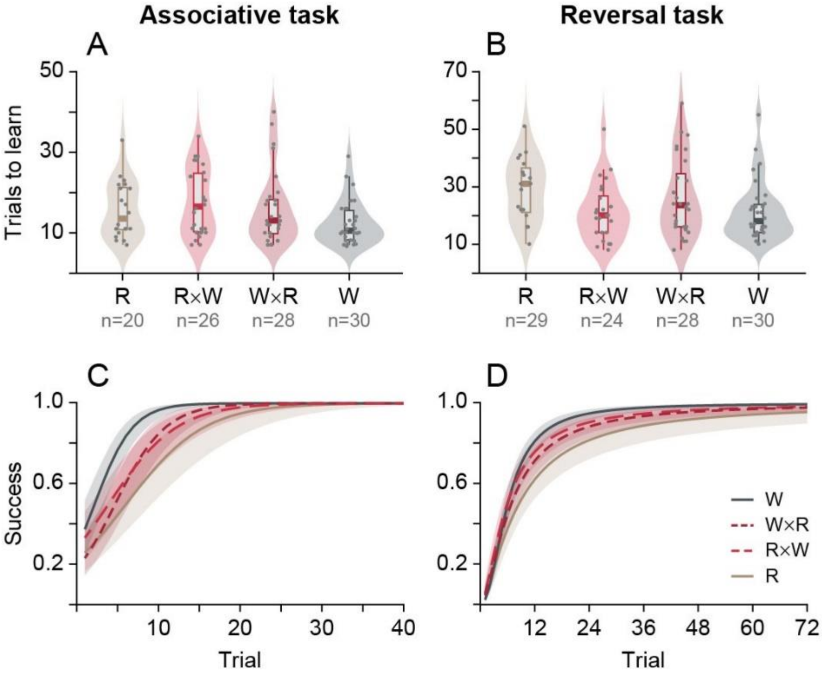

Figure 2. Performance of female fish from the two parental species (R; W) and the two F1 hybrid crossings (R×W; W×R) in the associative learning (left panels) and reversal learning (right panels) tasks. (a, b) Number of trials taken to learn each task. (c, d) Probability of correct choice over trials (lines show predicted model outputs and shaded areas indicate 95% confidence intervals). Points in (a, b) represent individual fish.

Additionally, we found that fish in the W×R group alone showed a positive correlation between trials needed to learn the association and trials needed to learn the reversal task (Fig. 3; Table S1c). The number of trials to learn the reversal task, but not the learning rate, differed between testing blocks (Table S1c,d). Individual females differed in their initial persistence to the previously learnt response (random intercept for Fish Identity; Table S1d) and in their speed of acquisition over trials (random slope for Fish Identity; Table S1d).

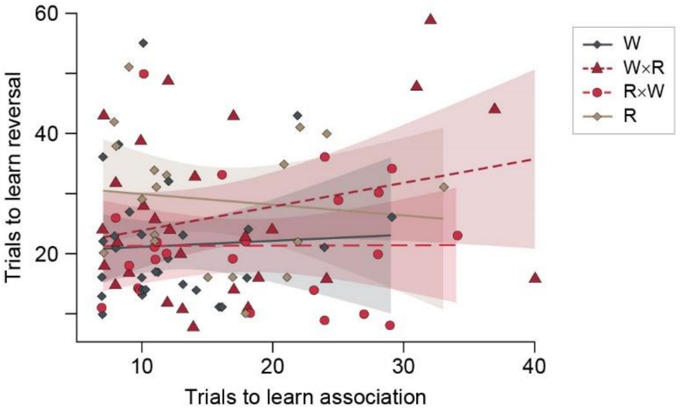

Figure 3. Relation between individual performance in the associative learning and the reversal learning task, showing a positive correlation for fish in the W×R group alone (triangles, short-dash line). Each point represents one fish.

Females in this study showed a naïve preference for the red discs; fish that were assigned red as a positive stimulus during the initial discrimination were faster to reach criterion and had a steeper learning rate (Table S1a,b). Similarly, fish initially trained to yellow and with red as the rewarded stimulus in the reversal task took less trials to succeed in the reversal and had a steeper learning rate (Table S1c,d).

3.2. Are hybrid phenotypes over-dispersed compared to parentals?

The hypervolume of 'cognitive space' describing phenotypic dispersion was similar between the four groups, with W×R hybrids showing a slightly larger dispersion value and common guppies (R) showing the least dispersion (Fig. 4a,b; Table S4). The simulated hybrid populations had consistently lower dispersion, with 95% KDEs having half the hypervolume of our four experimental groups (Fig. 4d; Table S4).

3.3. Are hybrid phenotypes transgressive?

The frequency of hybrid individuals found outside parental ranges, considered as transgressive, was of 17% for R×W and 14% for W×R individuals, corresponding to a total of eight transgressive hybrids (four from each group; Fig. 4a,b; Table S4).

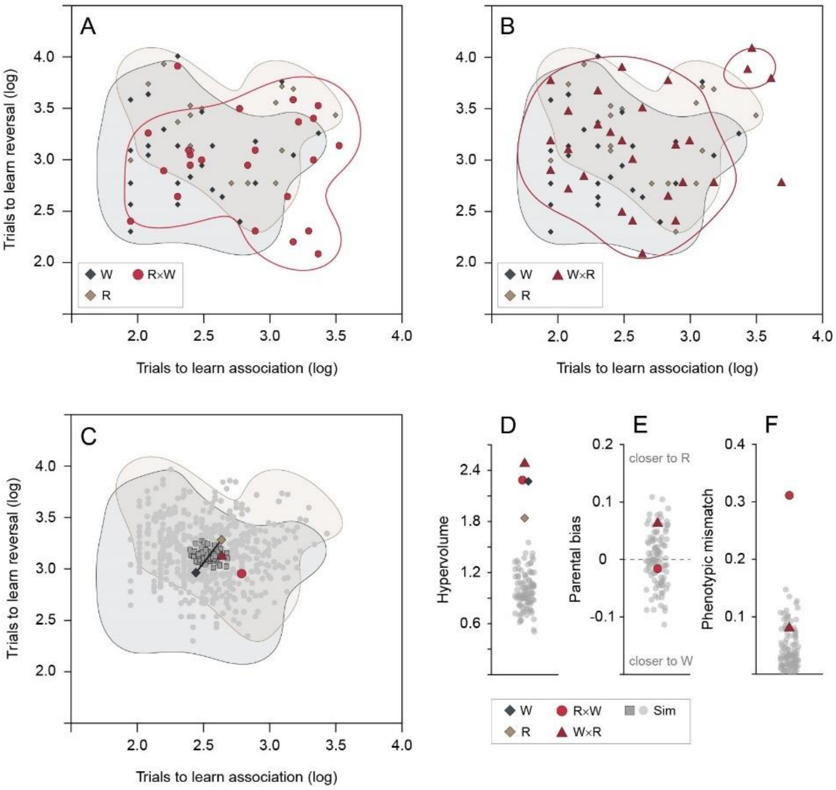

Figure 4. Distribution of individual fish in a two-dimensional 'cognitive space'; comparison of parentals (diamonds, shaded 95% KDEs) and **(A)** R×W hybrids (circles), showing four transgressive R×W fish; and **(B)** W×R hybrids (triangles), showing four transgressive W×R fish. **(C)** Position of

the mean phenotype of the four groups and of simulated hybrid populations (grey), in relation to the parental 95% KDEs. The black line connects parental mean phenotypes. **(D)** Hypervolume of observed and simulated 95% KDEs (phenotypic dispersion). **(E)** Distance of observed and simulated hybrid phenotypes from the midpoint between the parentals (parental bias). **(F)** Deviation of observed and simulated hybrid phenotypes from the line connecting parental mean phenotypes (phenotypic mismatch).

3.4. Is transgression linked to a deviation of the mean hybrid phenotype?

We found that ‘parental bias’ was low since the projection of the mean phenotypes of R×W and W×R was positioned between the two parentals and overlapped with the simulated hybrid populations (Fig. 4c,e; Table S4). However, R×W hybrids showed high phenotypic mismatch with parentals, since the mean phenotype of R×W deviated away from the line connecting parental mean phenotypes (Fig. 4c), with a value higher than any hybrid population simulated as a linear combination of parental phenotypes (Fig. 4f; Table S4). The mismatch of R×W was observed towards poor performance in associative learning but slightly better performance in reversal learning (Fig. 4a,c).

4. Discussion

We experimentally investigated how hybridization impacts cognitive abilities in first-generation reciprocal crosses between two closely related Poeciliid species. We found that both hybrid crosses showed mean learning scores that were intermediate between the parentals. However, hybrid phenotypes had slightly higher dispersion relative to parents, some hybrid individuals were transgressive, i.e., showed trait combinations outside the parental ranges, and the mean phenotype of one hybrid group deviated away from the axis of variation of the parental species.

Interspecific hybridization has regained recognition as an important source of phenotypic and genetic variation [8, 9, 11]. Such variation may enlarge the working surface for natural selection and promote the evolutionary potential of hybrid populations [13, 44]. For example, some hybrids of African cichlids or Galapagos finches show greater variation in morphological and/or behavioural phenotypes and express transgressive traits that allow them to explore ecological opportunities unavailable to the parentals [14, 24, 25]. Here, we found that cognitive phenotypes of F1 hybrids were slightly over-dispersed compared to parentals and that some individuals among the hybrids had transgressive combinations of learning scores. In addition, R×W hybrids showed a significant deviation away from the axis of variation of the parentals. We therefore suggest that hybridization may generate variation and transgression in cognitive abilities. These experimental results, even if just on first-generation hybrids, provide an important first test of whether hybridization can promote cognitive variation, a prerequisite for hybrids to have cognitive innovation potential [13, 24].

We found that only R×W hybrids showed a phenotypic mismatch with the parentals, while the reciprocal crossing (W×R hybrids) did not deviate from a linear combination of parental phenotypes. Asymmetries between F1 hybrid lineages in the degree of viability or sterility have been extensively documented in plants and animals [45-47]. Hybrid asymmetries typically arise from incompatibilities involving uniparentally inherited genetic factors (from mitochondria, chloroplasts, maternal transcripts, or sex chromosomes) and tend to be more pronounced in the heterogametic sex [45, 46]. In *Drosophila* fruit flies, hybrid males (the heterogametic sex) show greater impairment of foraging behaviour [6], and in wild *Poecile* chickadees the females (heterogametic sex in birds) show poorer cognitive skills [28]. The asymmetry we found between R×W and W×R hybrids hints at two general features of the genetic architecture of the traits we measured. First, the R×W mean phenotype significantly deviated from simulated linear combinations of the parental phenotypes, implying that additive inheritance, one of the primary causes of transgression [16-18, 20], is unlikely to regulate expression of the traits we measured. Second, the observed asymmetry between reciprocal crossings suggests the contribution of uniparentally inherited genetic factors. However, the genetic mechanism underlying extreme trait expression cannot be conclusively determined from phenotypic distribution alone and so we refrain from speculating further. Despite examining reciprocal hybrids, our study tested only females which are the homogametic sex in *Poecilia* guppies. Future work should compare cognitive performance of male and female reciprocal hybrids to test if greater reciprocal asymmetries are observed in males compared to females.

When comparing the learning performance of hybrids and parentals in associative and reversal learning independently, the mean phenotype of both hybrid groups was intermediate between the parentals. Intermediate phenotypes at the group level might indicate sub-optimal performance and decreased chances that hybrids escape competition from the parentals [7, 15, 28, 48]. However, it is possible that intermediate or poor hybrid phenotypes appear in early generations, but performance is rescued in later generations [15]. Further studies should compare the performance of F1 hybrids with that of F2 and later generations, to test how the expression of these traits varies after a few generations of recombination. Additionally, comparing F1 to later hybrid generations would indicate if transgression of F1 hybrids is due to heterosis and therefore transient, or if hybrid phenotypic variation in cognitive abilities may be heritable [16, 32]. Using experimental hybrids reared in similar conditions in the lab and later tested as adults, as in our study, can help to disentangle genetic effects from selection or plasticity effects, as opposed to using wild hybrids [28].

The phenotypic variation generated by hybridization can potentially enlarge the working surface for selection. However, the functional relevance and potential benefits of the transgressive trait values detected in this analysis are mostly unknown. Some studies indicate that higher learning scores, including associative and reversal learning, are linked with survival and other fitness benefits [49-52], but a few studies find the opposite pattern. For example, pheasants *Phasianus colchicus* that were slower to reverse a learnt association were more likely to survive when released into the wild [53]. In addition, cognitive investment may be linked to other costly functions or traits, and therefore better or poorer learning abilities may not linearly map into fitness benefits or costs [26, 50, 54]. A promising direction of research is to assay the same hybrid individuals in a battery of tasks testing different cognitive traits and functional behaviours, integrated with additional fitness-related traits such as viability and survival, growth, and reproduction.

Surprisingly, common guppies– frequently used as a test species in cognitive research [35, 38, 39, 55] – showed worse performance in both tasks compared to Endler's guppies and their hybrids. It is possible that differences in the ability to perceive and discriminate yellow from red between the four groups may have contributed to the observed cognitive differences between groups. Perception is an important cognitive process, together with learning and memory [56], that is relevant to the learning tasks we used. Colour vision is achieved by comparing signals from cone cells with differences in wavelength sensitivity, which is largely determined by opsin proteins [57]. Common and Endler's guppies both have three cone cells sensitive in the long-wavelength range (i.e., the green, yellow, and red portion of the visible light spectrum); the two species also have similar genetic architecture of long-wavelength sensitive opsin genes [58], indicating they have similar 'machinery' for colour vision in the yellow and red spectrum. However, as the two species may have differential expression of opsin genes, they may show differences in the ability to discriminate yellow and red [59]. To disentangle to what extent the cognitive differences we found are due to differences in perception or learning and memory, other colour combinations and tests of behavioural sensitivity to those [59] would be necessary. Inbreeding depression in our laboratory population of guppies could also be a possible explanation for the poorer performance of the common guppy. However, we think this is unlikely as guppies seem resilient to inbreeding depression [60] and the performance of the animals in this study falls within the learning rates of other studies using similar tasks and training procedures on different lab populations [35, 38, 39, 55]. Differences in foraging motivation, food perception, or physical abilities between the four groups of fish are also unlikely to explain our results since we initially pre-trained all fish to dislodge a single green disc and found no differences between groups in pre-training success.

Methods to quantify transgression and phenotypic dispersion vary across studies. Transgression is sometimes measured from individual hybrids in comparison to the range of values occupied by the parentals [16, 21, 25] or defined from the mean phenotype of the hybrid group [15, 61]. Our methodology allows for a combination of the two measures, resulting in a more comprehensive test of functional transgression applied to multidimensional cognitive phenotypes. Importantly, this method can be easily extended to other studies in ecology and evolution that aim to compare multivariate phenotypes between species or populations.

In conclusion, the results we present here indicate that hybridization may promote phenotypic variation and transgression in cognitive abilities, even when mean hybrid phenotypes are intermediate between the parentals. These results are an important step towards understanding the potential role of hybridization in promoting cognitive novelty and fuelling cognitive evolution.

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